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Concentrations and biological half-life of radioactive cesium in epigeic earthworms after the Fukushima Dai-ichi Nuclear Power Plant accident

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ABSTRACT

To understand the long-term behavior of radiocesium in the biological processes of a forest ecosystem, its concentration in Japanese epigeic earthworms (Megascolecidae), litter, and soil, and the ambient dose equivalent rates, were investigated after the Fukushima Dai-ichi Nuclear Power Plant accident. The metabolism of radiocesium in the earthworms was also investigated in the laboratory, and its biological half-life (T_b) was estimated. The concentration of ^{137}Cs in the habitat soil and litter changed from 2014 to 2016, with levels in the litter going from 44.9 Bq/g dw (in 2014) to 45.3 Bq/g dw (2015) and 10.7 Bq/g dw (2016); in soil, these values were 9.79 Bq/g dw, 7.14 Bq/g dw and 18.0 Bq/g dw, respectively. By contrast, no significant changes were observed in the concentrations in the earthworms, which were 4.87 Bq/g fw, 5.30 Bq/g fw and 4.67 Bq/g fw in 2014, 2015, and 2016, respectively. The ambient dose equivalent rates at the sampling site declined significantly over these three years, going from 2.15 μSv/h to 1.68 μSv/h and 1.35 μSv/h, mostly corresponding to physical decay of radiocesium. The majority (95%) of the ^{137}Cs in the earthworms, observed via autoradiography, was concentrated primarily in the intestine. The clearance of ^{137}Cs from the earthworms was described by dual exponential functions: the half-life in the rapid loss due to gut clearance was 0.10 days and a second slower loss due to physiological clearance was 27.4 days.

1. Introduction

The nuclear accident at the Tokyo Electric Power Company's Fukushima Dai-ichi Nuclear Power Plant (hereafter referred to as FDNPP) in March 2011 caused a serious release of radionuclides into the environment. Fukushima Prefecture is covered with 975,000 ha of forests, which accounts for approximately 71% of the total land area of the prefecture (Fukushima Prefectural Government, 2017). As a result, most of the radionuclides were captured in forest ecosystems (Hashimoto et al., 2012).

Radiocesium has long-term influence on the forest ecosystems due to its relatively long half-life (^{134}Cs: 2.06 y, ^{137}Cs: 30.1 y). After the accident, radiocesium was deposited directly on the floor of the forest (Koarashi et al., 2012) and accumulated further via throughfall, litterfall, and stemflow processes (Kato et al., 2012; Teramage et al., 2014; Loffredo et al., 2014). The annual vertical migration of ^{137}Cs to a depth of 10 cm in the soil only accounts for 0.1% of the total ^{137}Cs inventory (Nakanishi et al., 2014). As observed after the Chernobyl accident (Tikhomirov and Shcheglov, 1994; McGee et al., 2000), a large part of the radiocesium accumulates on the soil surface, including in the litter, in the long term. The radiocesium circulates in the forest ecosystem via biological cycles such as reabsorption by trees, retention by fungi, and direct ingestion by organisms. Therefore, it could be bioavailable for long-term transfer, for example, the detritus food web. The latter has been suggested to be a primary pathway of radiocesium (Murakami et al., 2014; Tanaka et al., 2016; Ishii et al., 2017), which means that detritivores are important as long-term carriers of radiocesium in a forest ecosystem.

Earthworms contribute to the disturbance of soil as ecosystem engineers (Lavelle et al., 1997) and are an important food resource for higher trophic consumers such as arthropods, birds, mammals, etc. It has been suggested that detritivores, including earthworms, supply radiocesium from the highly contaminated forest floor to consumers through the food web (Murakami et al., 2014). Earthworms can,

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therefore, contribute to the circulation of radiocesium in the soil and its transfer to organisms through the food web.

The results of a field survey conducted after the Chernobyl accident, to assess the effects of radiation on non-human biota, demonstrated catastrophic effects in soil invertebrate communities (including earthworms) (IAEA, 2006). Soil invertebrates had higher absorbed dose rates than other animals because their habitats were highly contaminated. This decreased the population densities and species diversity of soil invertebrates with an absorbed dose higher than approximately 30 Gy; the population densities of earthworms, in particular, decreased due to their high radio-sensitivity during the egg and juvenile stages (Krivolutzkii et al., 1992). This is why the International Commission on Radiological Protection selected the earthworm as a reference animal to assess the effects of radiation on the environment (ICRP, 2008).

For these reasons, earthworms are an important species for predicting the long-term environmental behavior of radiocesium and assessing the effects of radiation on non-human species. However, only a few field studies have been conducted on earthworms after the FDNPP accident. Moreover, there are a lack of studies on the biokinetics of radiocesium on the family Megascolecidae, which includes most of the species in Japan (Ishizuka, 1999).

Epigeic earthworms that live in the litter layer and the soil surface layer are an important ecological group discerning the behavior of radiocesium, which mostly accumulates in the ground surface layer. Hasegawa et al. (2013) conducted a field study on different species of epigeic earthworms in Fukushima and reported that earthworms from ecological groups of epigeic have similar radiocesium concentrations because of their similar habitats and physiological characteristics. In the present study, therefore, we focused on epigeic earthworms at family level to understand the behavior of radiocesium in a forest ecosystem.

The objectives of this study are to understand the temporal changes in the radiocesium concentration in earthworms from the mountainous forests of Fukushima and to examine the biokinetics of radiocesium in these earthworms. We conducted a field survey in Fukushima and compared the chronological changes in radiocesium in earthworms, litter, soil, and the ambient dose equivalent rates from 2014 to 2016. We also observed the distribution of radiocesium in the earthworms' bodies using autoradiography and determined the radiocesium concentrations in their body wall muscles, guts, and the other organs. Finally, we conducted clearance experiments of ^{137}Cs on the earthworm to estimate its biological half-life.

2. Materials and methods

2.1. Sampling site

The sampling site is an area of relatively high contamination located 40.1 km northwest of the FDNPP (latitude: 37°41′35″ N, longitude: 140°44′08″ E; Fig. 1). The initial deposition density of total radiocesium (^{134}Cs + ^{137}Cs) at the sampling site estimated from airborne monitoring survey was 1–3 MBq/m^2 (MEXT, 2011). The landscape is hilly and mountainous area, with agricultural fields and residential areas surrounded by mountainous forests. Residents were not permitted to live in this area during the study period from 2014 to 2016. The sampling site is a mixed forest dominated by deciduous broad-leaved trees and had not been decontaminated at the time of sampling. It had a radius of about 30 m (an area of ca. 2800 m^2), and the survey was conducted at the same site throughout the three years of the study.

2.2. Measurement and estimation of ambient dose equivalent rates

Ambient dose equivalent rates were measured using a NaI scintillation survey meter (TCS171, Hitachi, Ltd., Japan) which calibrated by Hitachi Aloka Medical, Ltd. on November 18, 2011. The calibration was carried out with the replacing method of JIS Z 4511–2005, using the

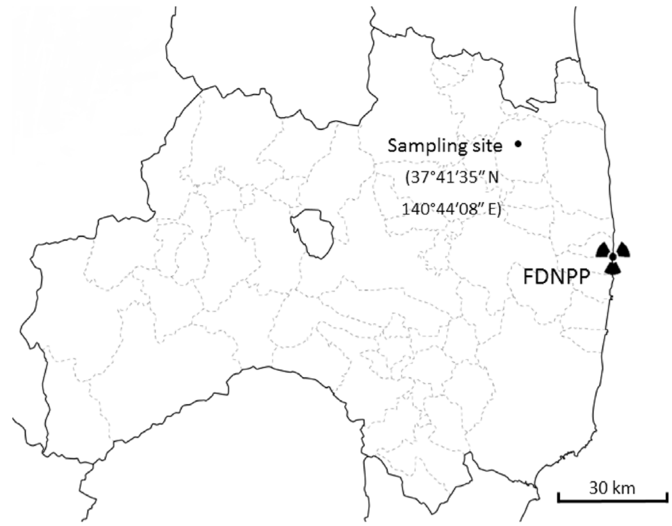

Fig. 1. Map of the sampling site and the Fukushima Dai-ichi Nuclear Power Plant (FDNPP).

working standard instruments (JCSS) irradiation apparatus. The measurement were conducted multiple points on the sampling site, 1 m above the ground and at least 20 m apart. To estimate these weathering of radiocesium from the sampling site, the ambient dose equivalent rates were compared to the estimated ambient dose equivalent rates from the physical half-life of radiocesium. For physical decay correction of ambient dose equivalent rates, the contribution ratio of ^{134}Cs and ^{137}Cs to the ambient dose equivalent rates were calculated using a conversion factor (IAEA, 2000) which assumed that the radiocesium was distributed uniformly on the ground. This value was applied with the ^{134}Cs/^{137}Cs activity ratio set to 1 from 2011.

2.3. Sampling and pretreatment of soil, litter, and earthworm

The soil was collected once at depths of 0–5 cm using a core sampler (50 mm in diameter, 51 mm high), and litter was sampled once in 25 cm × 25 cm area of the forest floor. The number of soil and litter samples taken in 2014, 2015 and 2016 were 1, 3, and 3, respectively. After being oven dried at 105 °C, the soil was screened through a 2 mm square mesh, and the litter was homogenized in a blender. The epigeic earthworms were collected by hand from the litter layer and soil surface (≤5 cm) from August to September. There were 1, 5, and 5 earthworm in five samples taken from 2014, 2015, and 2016, respectively; thus, 5, 25, and 25 individuals were collected, respectively, in those years. An additional 75 earthworms were collected in 2016 for the clearance experiments. The earthworms were identified as belonging into the family Megascolecidae from the external shape of their bodies, particularly the clitellum. In addition, the internal shapes were observed after the dissection of a few individuals. To measure the ^{137}Cs accumulation in each part of the body, 25 earthworms were dissected and the intestines, body wall muscles, and other organs were separated. The ^{137}Cs concentrations in the earthworms were determined from data including gut contents, since predators would consume the entire body of the earthworm (Sheppard et al., 1997); this method is therefore appropriate for understanding how radiocesium is transferred through the food web.

2.4. Measurement of ^{137}Cs in the samples

The soil and litter samples and the earthworms were packed into plastic containers (U-8: 47 mm diameter, 60 mm in height). The retention curve of ^{137}Cs in the earthworms, which were packed individually in plastic petri dishes (60 mm in diameter, 15 mm high), were determined for estimating the biological half-life.

The activity of ^{137}Cs (662 keV) was determined by the gamma-ray spectrometry using a high-purity germanium detector (GEM30-70, ORTEC, USA) with a multi-channel analyzer (Easy-MCA-8k, ORTEC, USA). The counting efficiency of the detector for U-8 containers was calibrated using a U-8 volume source of ^{137}Cs (24FY039, Japan Chemical Analysis Center). The counting efficiency for petri dish was calibrated by the KCl method, which used an efficiency curve normalized by the gamma-ray peak for 1460 keV from ^{40}K in KCl placed in the same type of vessel (Fujiwara et al., 2015). Each sample was measured for approximately 10,800–27,000 s. The radioactivities of the litter and soil samples were obtained as Bq/g dry weight (Bq/g dw); that of the earthworms were obtained as Bq/g fresh weight (Bq/g fw).

2.5. Autoradiography

The autoradiography of the earthworms collected in 2014 and 2015 was performed using high-resolution imaging plates (BAS-IP MS 2025E, Fujifilm, Co., Japan.) exposed for 8 day at −80 °C after contact with the plate. The autoradiographic images on the plate were read with a scanner (Typhoon FLA7000, GE healthcare, Japan Co.).

2.6. Estimation of the biological half-life of ^{137}Cs in the earthworms

The earthworms collected in 2016 were moved from the contaminated sampling site media to a non-radioactive media for determining the ^{137}Cs retention. Concentration activity of five individuals was measured at 1, 3, 6, 12, 24, 48, 120, 240 and 360 h after the moving. Concentration of ^{137}Cs at each time point was defined as median value in the five earthworms. To estimate the biological half-life (T_b) of ^{137}Cs in these earthworms, the following double exponential equation was applied (Eq. (1)).

$$A_t = A_{0f}\, e^{-\lambda_f t} + A_{0s}\, e^{-\lambda_s t} \tag{1}$$

where A_t is concentration of ^{137}Cs (Bq/g) at time t, A_0 is the initial concentration of ^{137}Cs (Bq/g) ($t = 0$), and λ_f and λ_s are the elimination rate constants for the fast and slow clearance, which caused by simple passage through intestine and physiological clearance of assimilated ^{137}Cs, respectively. Log-transformed data were used to estimate A_0 and λ using the non-linear least squares fitting method.

2.7. Statistical analyses

To determine the changes in the ambient dose equivalent rates and the concentration of ^{137}Cs in the samples, the differences in the values between the years were analyzed using the Kruskal–Wallis test in R version 2.15.3 (R Core Team, 2013).

3. Results

3.1. Ambient dose equivalent rates at the sampling site

The ambient dose equivalent rates measured 1 m above the ground at the sampling site are shown in Fig. 2 and Table S1 in supplementary materials. The median values show a significant and consistent decrease, from 2.15 μSv/h to 1.35 μSv/h, between 2014 and 2016 (Kruskal–Wallis test, $p < 0.001$); these decreased by 36% from 2014 to 2016. All the measurement data were provided in Table S2. The ambient dose equivalent rates in 2014 decreased to 1.77 μSv/h and 1.55 μSv/h in 2015 and 2016, respectively, by the physical decay of radiocesium; those values were slightly higher than actual ambient dose equivalent rates at the sampling site (Fig. 2).

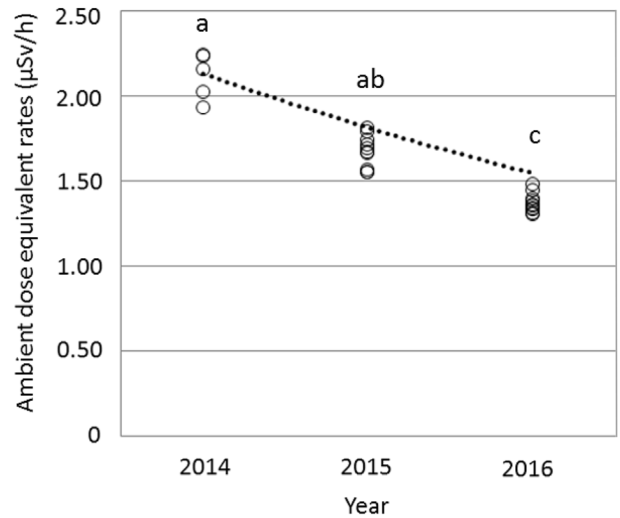

Fig. 2. Ambient dose equivalent rates at 1 m above ground at the sampling site, which significantly decreased from 2014 to 2016 (Kruskal–Wallis test, $p < 0.001$). Different letters above the plots denote significant differences in multiple comparisons by Scheffe's method ($p < 0.05$). The broken line is the decline of ambient dose equivalent rates in 2014 by the physical decay of radiocesium.

3.2. Chronological changes in ^{137}Cs in field samples

The concentration of ^{137}Cs in the litter was 44.9 Bq/g dw in 2014, increased only slightly in 2015 (45.3 Bq/g dw), and then dropped to 10.7 Bq/g dw in 2016 (Table S2). The concentrations of ^{137}Cs in the soil were 9.79 Bq/g dw, 7.14 Bq/g dw, and 18.0 Bq/g dw in 2014, 2015, and 2016, respectively. Apparent tendency was not observed in the concentrations of ^{137}Cs in the litter and soil over the study period. In contrast, the median value of ^{137}Cs in the earthworms from 2014 to 2016 did not change significantly; the values for 2014, 2015, and 2016 were 4.87 Bq/g fw, 5.30 Bq/g fw, and 4.67 Bq/g fw, respectively (Kruskal–Wallis test, $p = 0.878$; Fig. 3).

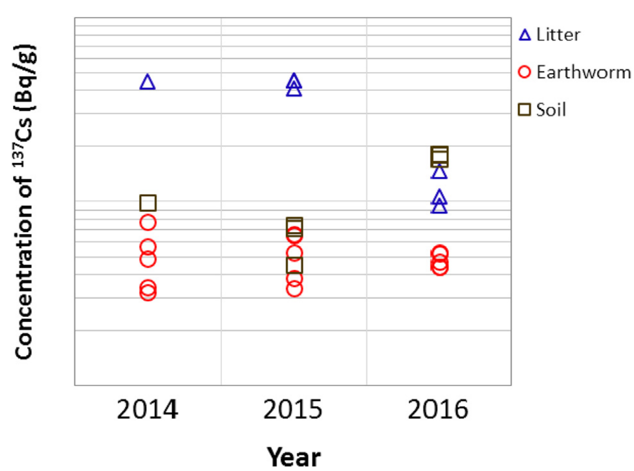

Fig. 3. Changes in the ^{137}Cs concentration in the litter, earthworms, and soil from 2014 to 2016. Triangles, circles and squares represent the litter, earthworms and soil respectively. The concentration of ^{137}Cs in the litter and soil were determined from the dry weight; that of the earthworms was determined from the fresh weight. The concentration of ^{137}Cs in the earthworms did not vary significantly with time after the accident from 2014 to 2016 (Kruskal–Wallis test, $p = 0.878$; multiple comparisons using Scheffe's method, $p < 0.05$).

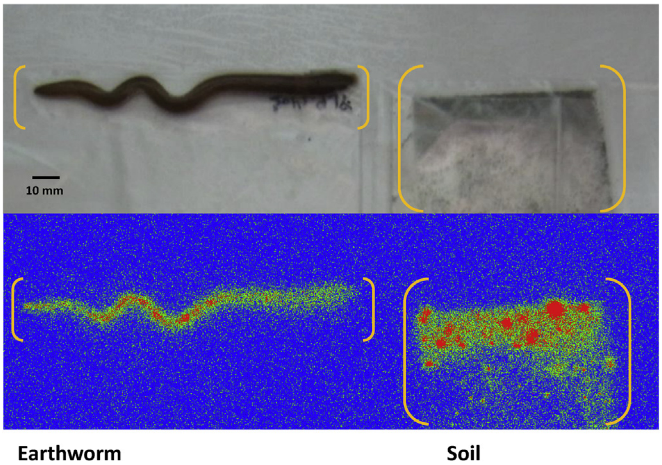

Fig. 4. Autoradiographs (below) and photographs (above) of an earthworm and habitat soil. The earthworm was exposure in a fresh condition, and the soil (≤5 cm) was in a dry condition for spread on the plate. Red and green areas indicate high and low concentrations, respectively, of radiocesium; blue areas indicate the background concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Autoradiography

Autoradiographs of the earthworms showed that radiocesium was distributed mainly in the digestive tract along the centerline of their bodies (Fig. 4). The autoradiograph taken after the dissection indicated lower accumulations in the body wall muscles and other organs and higher concentrations in the intestines (Fig. 5).

3.4. Concentrations of ^{137}Cs in each part of earthworms

The concentrations of ^{137}Cs in the intestines, body wall muscles, and other organs in the earthworms were determined using a germanium semiconductor detector. The median concentrations of ^{137}Cs in these organs were 6.48 Bq/g fw, 0.17 Bq/g fw, and 0.10 Bq/g fw, respectively (Fig. 6). The ratio of the ^{137}Cs concentration in the body wall muscles to that in the intestines was 0.02. The percentages of ^{137}Cs in the earthworm intestines, body wall muscles, and other organs were 95%, 2.6% and 1.4%, respectively, of the total retained ^{137}Cs.

3.5. Estimating the biological half-life of ^{137}Cs

As shown in Fig. 7, the clearance curve of ^{137}Cs from the earthworm body seemed to be bi-phasic (i.e., it had a two-component clearance).

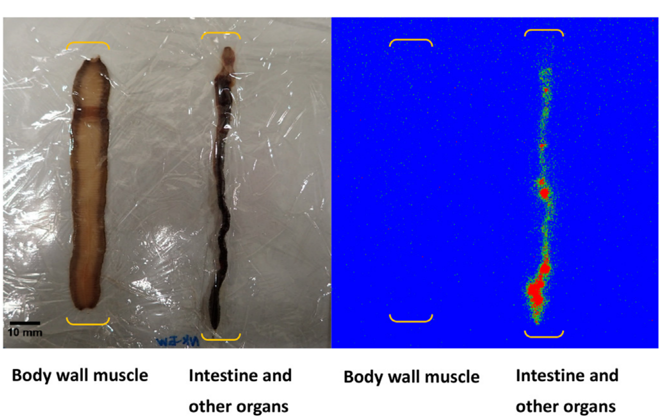

Fig. 5. Autoradiographs (right) and photographs (left) of a dissected earthworm for the body wall muscles and intestine and other organs.

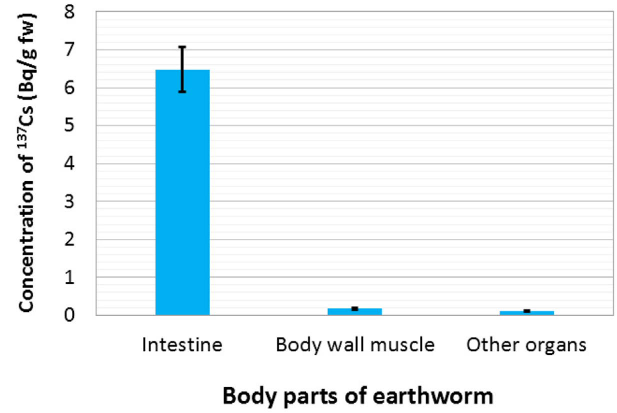

Fig. 6. Concentrations of ^{137}Cs in each part of dissected earthworms. The error bars indicate standard deviations.

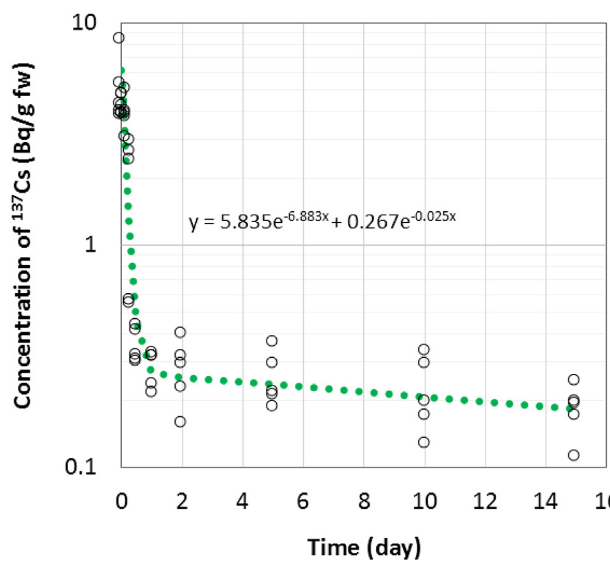

Fig. 7. Clearance of ^{137}Cs from the earthworm body.

By fitting the curve to a double exponential equation (Eq. (1)), the half-life of the first phase (T_b fast) was calculated as 0.10 days. The second phase of clearance was slower with a half-life (T_b slow) of 27.4 days. The fast and slow phases were estimated to have cleared 95% and 4.3%, respectively, of the total retained ^{137}Cs (Table S3).

4. Discussion

The decline of ambient dose equivalent rate in 2014 by physical decay of radiocesium is plotted with a dotted line in Fig. 2, showing that the decline curve is only slightly higher than the measured doses in 2015 and 2016. This suggests that the downward migration and/or runoff of radiocesium was not so large at the sampling site. This finding coincides well with previous studies that report extremely slow downward migration and low-runoff of radiocesium from the floor of deciduous forests in Fukushima after the accident (Nakanishi et al., 2014; Niizato et al., 2016). Kinase et al. (2014) estimated that the ecological half-life of radiocesium is much longer in deciduous forests than in other land areas. It may possibly be reasonable to assume that the temporal changes in the ambient dose equivalent rates found in this study were attributable mainly to the physical decay of radionuclides, although the precise mechanisms are not readily apparent from this study, and that the radiocesium was retained in the forest floor during the survey period. There is also the possibility that the concentration of radiocesium in the litter decreased during the study period, while that in the soil increased, although there are too few samples to determine this and the tendency is not consistent over the years (Fig. 3). Rapid downward migration of radiocesium from the litter layer to the topsoil was observed in deciduous forests in Fukushima in other studies (Fujii et al., 2014), and similar findings was also reported for the period 2013–2015, with large seasonal variations due to decomposition and input from contaminated litter fall (Takada et al., 2017). Therefore, the observed changes in the ^{137}Cs concentration in the litter and soil might represent a movement of ^{137}Cs on the forest floor due to decomposition and migration from litter to the topsoil.

In contrast, it is interesting that no significant changes were observed in the earthworms during the study period. They move into the litter and the surface of the soil during feeding and egestion, therefore homogenizing the radiocesium on the forest floor (Tyler et al., 2001; Jarvis et al., 2010). This role of earthworms as bioturbators could be one reason why the ^{137}Cs concentrations in the earthworms did not so much change. The radiocesium in the earthworm may have shown as value of the contamination of the organic layer in the forest, which has been shown to distribute unevenly (Koarashi et al., 2014).

The earthworms' feeding habits could provide another explanation for lack of yearly change in the ^{137}Cs concentration in their bodies. The radiocesium is kept in the organic horizon of forest soils by biological processes such as microbial immobilization and recycling (Brückmann and Wolters, 1994), and the long-term retention of radionuclides in these layers have frequently been attributed to microbiological activity (Steiner et al., 2002). Soil microorganisms are major known food sources for earthworms, and protozoa and fungi are assumed to form a substantial part of them (Edwards and Fletcher, 1988; Brown, 1995; Bonkowski and Schaefer, 1997). Moreover, the earthworms have been reported as selectively feeding on microorganisms (Doube et al., 1997; Bonkowski et al., 2000; Neilson and Boag, 2003). The epigeic earthworms, Megascolecidae, fed more on organic materials than other ecological groups as anecic and endogeic earthworms (Uchida et al., 2004). Therefore, it is apparent that the earthworms collected in this study consumed soil microorganisms, which could be a reason why the radiocesium concentrations in their bodies did not change so much. It is worth noting that the zoological and ecological characteristics of earthworms may make them good indicators of the level of radiocesium contamination at surface of organic layer in forests.

The autoradiograph of the earthworm body showed that the radiocesium was distributed mainly in its intestines and not much in the other body parts (Figs. 4 and 5), and quantitative measurements with the germanium detector showed that 95% of the ^{137}Cs was located in intestine (Fig. 6). This indicates that most of the radiocesium ingested by earthworms are excreted through the digestive tract. Ninety-five percent of the total activity was cleared in the first rapid phase, with the T_b representing fast and mainly gut clearance, while 4.3% of the ^{137}Cs was cleared in the second, slower phase, with the T_b representing slow physiological clearance. These values correspond with the abundance ratio of ^{137}Cs in the earthworm, the majority of which is in intestine and the rest of which (4.0%) represented the values of the body wall muscle and other organs combined. Previous studies on the biological half-life of radiocesium in earthworms are summarized in Table 1; in this study, the T_b fast value (0.10 d) is a little faster than those from the other three studies, and the T_b slow (27.4 d) is within the range found by of Brown and Bell (1995).

The present study on the abundance ratio of ^{137}Cs in each part of the earthworm and biological half-life indicates that ^{137}Cs will not be highly bioaccumulated in the body wall muscle of the earthworm. Hasegawa et al. (2015) investigated the changes of radiocesium bioavailability in epigeic earthworms and reported a decrease in the initial phase after the accident from 2011 to 2013. Radiocesium was fixed strongly soil minerals time-dependently; therefore, the body wall muscle of the earthworms will not bioaccumulate radiocesium in the long-term.

The radiocesium concentrations in gut contents of the earthworms depends on the contamination levels of the habitat media. A large part of the radiocesium accumulates in the soil surface layer in forests over the long-term; this would maintain the concentrations of radiocesium in the earthworms. Therefore, detritivore species in the same habitat, including earthworms, are a likely primary transfer pathway for radiocesium through the food web.

Earthworms ingest 5–10% of the topsoil mass per year (Lee, 1985; VandenBygaart et al., 1998; Curry and Schmidt, 2007; Jarvis et al., 2010). Therefore, their feeding and egestion activities would contribute to disturbance of radiocesium in surface soil. Determination of the contribution of soil organisms to the movement of radiocesium in surface soil may help in understanding of the long-term behavior of radiocesium in the forest ecosystem. However, further studies are needed to elucidate the contribution of earthworms to the movement of radiocesium in surface soil.

The chronological changes in radiocesium levels in earthworms corresponded to the changes in contamination levels on the forest floor estimated from ambient dose equivalent rates. This suggests that earthworms could potentially be a very suitable indicator of radiocesium contamination in mountainous forested areas in Fukushima.

5. Conclusions

We conducted a field and laboratory investigation of Japanese epigeic earthworms to understand the long-term behavior of radiocesium in the biological processes of a forest ecosystem and to elucidate the biokinetics of the radiocesium in earthworms. Monitoring and analyzed data on the ambient dose equivalent rates at a sampling site suggests that the downward migration and/or runoff of radiocesium from the sampling site was not so large. This implies that radiocesium is retained on the forest floor. While the yearly change in concentration of ^{137}Cs in litter and soil did not show an apparent tendency, the correspond levels in earthworms remained stable during the study period. The concentration of radiocesium in the earthworms reflected the average value of the radiocesium that was not uniformly distributed in the organic layer on the forest floor. This may be a reason why the ^{137}Cs levels did not vary greatly in the earthworms, despite changes in the habitat media such as litter and soil. Therefore, epigeic earthworms could be a good bioindicator for radiocesium contamination in mountainous forested areas where radiocesium has been distributed heterogeneously.

A large part (95%) of the total ^{137}Cs was retained in the contents of

Table 1
Comparison of the biological half-life of radiocesium (^{134}Cs and/or ^{137}Cs) in this study and previous studies.

Family	Species	Ecological type	Culture media	Biological half-life (day)		References
				T_b fast	T_b slow	
Lumbricidae	*Lumbricus terrestris*	Anecic	Litter (Apple leaves)	0.16–0.33	25–54	Brown and Bell, 1995
Lumbricidae	*Lumbricus terrestris*	Anecic	Soil	0.20–0.27	15–26	Brown and Bell, 1995
Lumbricidae	*Lumbricus terrestris*	Anecic	Litter	1.4	24	Sheppard et al., 1997
Megascolecidae	Unknown	Epigeic	Litter and soil	0.10 ± 0.01	27.4 ± 16.0	This study

±: Standard error.

the earthworm intestines, while the distribution of the former in the body wall muscle and other organs was low (4%). The biological half-life of ^{137}Cs in the earthworms indicated that most of the radiocesium they ingested were excreted rapidly with half-life of 0.10 d. This means that the concentrations of radiocesium in earthworms reflect levels of contamination in their habitat. The results of autoradiography and biokinetic analysis of radiocesium in earthworm showed that radiocesium will not be highly bioaccumulated in their body wall muscle; however, their entire body (including the content of their guts) will contain some concentrations of radiocesium due to their ecological characteristics and highly contaminated habitat. This means that earthworms could be a primary pathway for long-term migration of radiocesium through food webs. Therefore, long-term monitoring of earthworms may contribute to further understanding of the behavior of radiocesium in the forest ecosystems where the biological migration of radiocesium is expected to be significant in the long-term.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvrad.2018.06.020.

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